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# Cellular Mechanisms of Neurogenic Inflammation

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**ABSTRACT**

Since the initial observations that stimulation of sensory neurons produces vasodilation, plasma extravasation, and hypersensitivity, much progress has been made in understanding the etiology of neurogenic inflammation. Studies have focused largely on the role of the neuropeptides, substance P and calcitonin gene-related peptide, which are released in the periphery by activation of small diameter sensory neurons. Recent work, however, has begun to emphasize the cellular mechanisms involved in regulating the release of proinflammatory substances from sensory neurons. In this perspective, discussion centers on a number of inflammatory mediators that activate various signal transduction pathways to augment excitability of and transmitter release from sensory neurons. Emphasis is placed on those pathways where multiple lines of evidence support their importance in initiating neurogenic inflammation. Recent studies, however, support the notion that there are novel compounds released during injury that can stimulate or sensitize sensory neurons. Furthermore, only now are intracellular signaling pathways that have been identified in other cell systems being studied in sensory neurons to establish their role in neurogenic inflammation. The challenge remains to ascertain the critical transduction pathways that regulate transmitter release from sensory neurons since this phenomenon triggers neurogenic inflammation. In addition, the cellular mechanisms involved in alterations in neuronal excitability during injury and the cellular pathways that maintain the inflammatory response over time need to be determined. With these advances, we will be able to develop therapeutic interventions to minimize deleterious consequences of neurogenic inflammation.

## Neurogenic Inflammation

More than a century ago, the first observations were made that activation of dorsal root ganglia neurons results in vasodilation, suggesting that these neurons not only conduct afferent information to the spinal cord, but also subserve an efferent function (Bayliss, 1901). Since that time, abundant evidence has accumulated supporting the notion that activation of peripheral terminals of sensory neurons by local depolarization, axonal reflexes, or dorsal root reflexes releases bioactive substances. These substances, in turn, act on target cells in the periphery such as mast cells, immune cells, and vascular smooth muscle producing inflammation, which is characterized by redness and warmth (secondary to vasodilation), swelling (secondary to plasma extravasation), and hypersensitivity (secondary to alterations in the excitability of certain sensory neurons). We call this phenomenon "neurogenic inflammation", that is, inflammatory symptoms that result from the release of substances from primary sensory nerve terminals.

Of major importance in the generation of neurogenic inflammation are the small diameter sensory neurons that are sensitive to capsaicin, the vanilloid found in hot peppers (for review, see Holzer, 1988). Intradermal injection of capsaicin rapidly produces hypersensitivity and flare, and these symptoms can be prevented by denervation or by preexposure to capsaicin, which presumably depletes neuropeptide content in the terminals. Additionally, destruction of capsaicin-sensitive fibers attenuates neurogenic inflammation produced by antidromic stimulation of sensory fibers.

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**ABBREVIATIONS:** SP, substance P; CGRP, calcitonin gene-related peptide; DRG, dorsal root ganglion; PKC, protein kinase C; PLC, phospholipase C; IP3, inositol 1,4,5-trisphosphate; DAG, 1,2-diacylglycerol; PAR, proteinase-activated receptor; CaM kinase, calcium/calmodulin-dependent protein kinase; $PGE_2$, prostaglandin $E_2$; $PGI_2$, prostaglandin $I_2$; TNF$\alpha$, tumor necrosis factor-$\alpha$; COX2, cyclooxygenase 2; 5-HT, 5-hydroxytryptamine; NO, nitric oxide; MAP kinase, mitogen-activated protein kinase.

Although there are a number of potential substances released from capsaicin-sensitive sensory neurons, most evidence supports the notion that the neuropeptides, substance P (SP) and calcitonin gene-related peptide (CGRP) are major initiators of neurogenic inflammation (for review, see Holzer, 1988). These putative neurotransmitters are located in a subset of small dorsal root ganglion (DRG) cells, which give rise to the lightly myelinated A delta and unmyelinated C fibers (Willis and Coggeshall, 1991). Stimulation of C fibers releases SP and CGRP peripherally and in the dorsal spinal cord, whereas destruction of capsaicin-sensitive fibers with neonatal capsaicin depletes the content of these peptides (Holzer, 1988). Substance P and CGRP produce symptoms of neurogenic inflammation by interacting with endothelial cells, mast cells, immune cells, and arterioles (see review by Maggi, 1995). These symptoms can be mimicked by administration of SP or CGRP agonists and attenuated by administration of antibodies directed against these peptides or by antagonists at their receptors (Maggi, 1995).

In addition to SP and CGRP, other substances such as glutamate and prostaglandins are synthesized and released from small diameter sensory neurons. The release of glutamate from central terminals of sensory neurons is well documented, but its peripheral actions and potential role in neurogenic inflammation are yet to be determined. Evidence also suggests that sensory neurons contain cyclooxygenases and are capable of synthesizing proinflammatory prostaglandins (Vasko et al., 1994). Because glutamate and prostaglandin receptors are localized on small diameter sensory neurons (Carlton et al., 2001; Donaldson et al., 2001; Southall and Vasko, 2001), it is intriguing to speculate that these substances have autocrine as well as paracrine actions when released. The questions remain as to what other potential mediators of neurogenic inflammation are released from capsaicin-sensitive sensory neurons and whether other types of sensory neurons contribute to the inflammatory symptoms.

Since release of substances from sensory neurons triggers neurogenic inflammation, knowing what conditions and chemicals regulate release and understanding the cellular mechanisms mediating release are critically important in being able to intervene during chronic inflammation. Indeed, the ability to regulate excitability of sensory neurons may have important therapeutic consequences in a number of diseases including (but not limited to) migraine, arthritis, chronic obstructive pulmonary disease, asthma, and inflammatory bowel disease. Consequently, the remainder of this perspective will focus on issues of the regulation of transmitter release from small diameter sensory neurons.

## Agents and Mechanisms That Increase Transmitter Release Directly

There are a number of substances and conditions that have been reported to activate sensory neurons and these are summarized in Table 1. Early evidence that these agents are excitatory was based largely on in vivo studies showing that their injection into various cutaneous sites or into vascular beds augmented nociceptive behaviors and enhanced firing of nociceptive sensory neurons. One limitation of these types of experiments, however, is that it is not possible to clearly establish whether the injected substance is acting directly on sensory neurons or is augmenting the production of other chemicals which in turn act on the neurons. This remains a significant problem given the number of cells and chemicals (known and unknown) that contribute to the inflammatory response. To address this issue, numerous investigators have developed the strategy of examining isolated sensory neurons either after acute dissociation or after growth in culture. Use of isolated sensory neurons provides a direct way of studying release and cellular mechanisms with minimal contamination by other cell types. One limitation, however, of using cell culture is the possibility that these cells do not reflect normal physiological processes because they exist in an artificial environment. By combining studies on isolated cells with in situ or in vitro experiments, however, confirmation of physiological relevance can be obtained. For the purpose of this perspective, we conclude that a substance has a direct stimulatory action on sensory neurons if 1) receptors for the excitatory substance are expressed on sensory neurons, 2) activation of these receptors increases calcium entry presumably by depolarization or activating currents in sensory neurons, and 3) exposing isolated sensory neurons to the substance stimulates the release of transmitters.

Of the inflammatory mediators listed in Table 1, evidence exists to demonstrate that capsaicin, heat, protons, bradykinin, and tryptase satisfy the above criteria. Capsaicin, heat, and protons activate the recently cloned VR1 receptor, which is localized on small diameter sensory neurons (Caterina et al., 1997, Tominaga et al., 1998). This activation results in the opening of a nonselective cation channel, increasing cal-



TABLE 1
Actions and cellular mechanisms of selected inflammatory mediators on excitability and neuropeptide release from sensory neurons

| Mediator | Depolarize or Elicit Currents | Directly Release Neuropeptides | Augment Evoked Release of Neuropeptides | Mechanism |
|---|---|---|---|---|
| Acetylcholine | Yes | ? | ? | Nicotinic receptors |
| ATP | Yes | No | Yes | P2 receptors/channels |
| Bradykinin | Yes | Yes | Yes | BK receptor-activation of $IP_3$, DAG/PKC |
| Capsaicin | Yes | Yes | ? | Vanilloid-gated channels |
| Cytokines | No | ? | Yes | Tyrosine kinases? |
| Glutamate | Yes | ? | ? | NMDA/AMPA/kainate/metabotropic receptors |
| Heat | Yes | Yes | ? | Heat-gated channels/VR1 |
| Hydrogen ions | Yes | Yes | ? | ASICs/VR1 |
| Nerve growth factor | No | No | Yes | TrkA receptors |
| Nitric oxide | No | No | ? | cGMP/G kinases |
| Prostaglandins ($PGE_2$, $PGI_2$) | No | No | Yes | EP, IP receptors cAMP/PKA |
| Serotonin | No | ? | ? | 5-$HT_3$ receptor |
| Trypsin, tryptase | Yes | Yes | ? | Proteinase-activated receptor 2 |

NMDA, *N*-methyl-D-aspartate; BK, bradykinin; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ASIC, acid-sensing ion channel.

cium entry both through this channel and through voltage-gated calcium channels activated by sodium-induced depolarization (Szallasi and Blumberg, 1999). These stimuli augment the release of the neuropeptides SP and CGRP from isolated sensory neurons, from a number of peripheral tissues, and from dorsal spinal cord (Bevan and Geppetti, 1994; Kessler et al., 1999; Vasko et al., 1999), and this release is dependent on the entry of calcium into the cells (see Fig. 1). There also is evidence that that the VR1 receptor is localized on endoplasmic reticulum and may contribute to the release of calcium from intracellular stores (Olah et al., 2001). The importance of these stores in transmitter release is yet to be determined.

In addition to activating vanilloid channels, thermal stimuli also are capable of activating other heat-sensitive cation channels in sensory neurons (Nagy and Rang, 1999). Protons also activate a family of acid-sensing ion channels, and a number of these are localized on various types of sensory neurons (Lingueglia et al., 1997; Chen et al., 1998). The role that activation of these channels has in neurogenic inflammation has not been established, despite the evidence that they are important in causing transmitter release.

The search continues for endogenous substances that activate VR1 receptors with recent work focusing on anandamide and other eicosanoids as putative endogenous agonists (Zygmunt et al., 1999; Hwang et al., 2000). A disparity exists, however, between the concentrations of these agents that activate their respective receptors to produce a number of biological responses and the concentrations necessary to activate VR1 currents. Furthermore, anandamide at low concentrations is antinociceptive and inhibits peptide release from sensory neurons (Richardson et al., 1998), which is inconsistent with VR1 agonist activity. Whether metabolic products of arachidonic acid or other molecules are found to be VR1 agonists, the discovery of endogenous ligands for the capsaicin receptor will be of major significance to understanding and regulating neurogenic inflammation.

In contrast to direct activation of ion channels, bradykinin stimulates transmitter release from sensory neurons (Geppetti, 1993) through activation of the protein kinase C (PKC) transduction cascade (Fig. 1). Bradykinin binds to the $B_2$ receptors on sensory neurons, and this results in the activation of phospholipase C (PLC) leading to an increase in inositol 1,4,5-trisphosphate ($IP_3$) and 1,2-diacylglycerol (DAG). Inositol 1,4,5-trisphosphate, in turn acts on $IP_3$ receptors to release calcium from intracellular stores, whereas DAG activates various isoforms of PKC (Burgess et al., 1989). Although bradykinin has also been reported to increase intracellular calcium (Thayer et al., 1988), the activation of PKC appears to be a major component of bradykinin-induced neuropeptide release since preexposure to inhibitors of PKC or down-regulation of PKC attenuates release (Barber and Vasko, 1996). Additionally, activation of PKC by phorbol esters results in an excitation of and an increase in neuropeptide release from sensory neurons grown in culture in a manner analogous to bradykinin (Barber and Vasko, 1996; Vellani et al., 2001). One conundrum, however, is the mechanism by which bradykinin stimulates transmitter release from sensory neurons. It is possible that activation of PKC by bradykinin causes depolarization of afferent fibers, but studies attempting to show this have yielded conflicting results. There also is the suggestion that bradykinin is not a direct activator, but lowers the threshold for activation of VR1 receptors by heat and thus allows cells to depolarize at temperatures that normally would not alter excitability (Liang et al., 2001).

Tryptase, an enzyme localized in mast cells, represents a novel mediator of neurogenic inflammation (Vergnolle et al., 2001). This enzyme binds to proteinase-activated receptor 2 (PAR2) where it cleaves the molecule to unmask an active N-terminal sequence of the receptor. Proteinase-activated receptors (PARs) are localized on a number of cells including small diameter sensory neurons (Steinhoff et al., 2000; Vergnolle et al., 2001). Tryptase-induced neurogenic inflammation is attenuated by neurokinin 1 receptor antagonists strongly suggesting that the effect of this inflammatory mediator is secondary to release of SP from sensory nerve endings. Furthermore, trypsin, tryptase, and PAR2 agonists in-

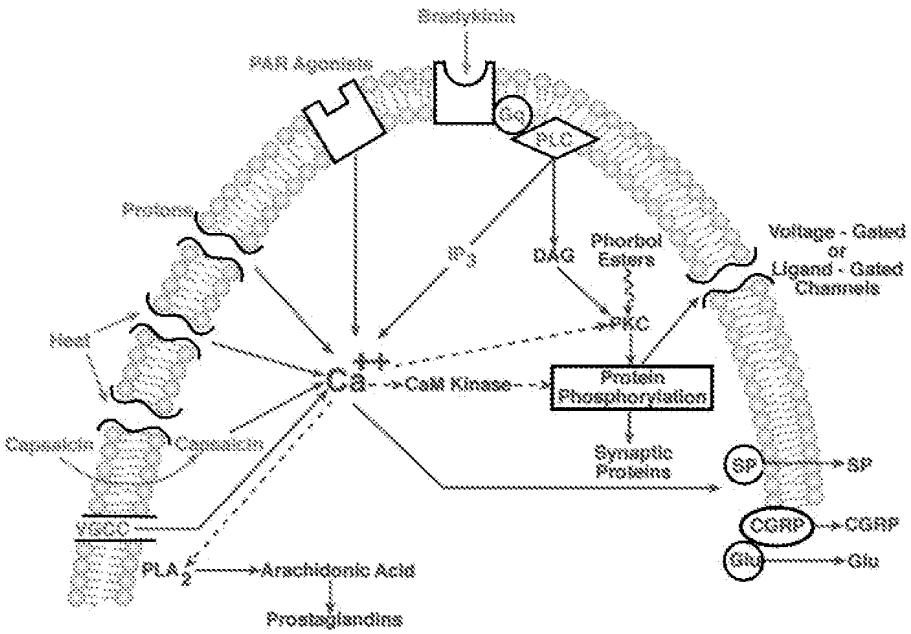


**Fig. 1.** Cellular pathways involved in transmitter release from sensory neurons. Capsaicin, heat, and protons activate ligand-gated cation channels that allow sodium and calcium to enter the cell. Any depolarization that results also activates voltage-gated calcium channels (VGCC) to further calcium entry. Bradykinin activates G-protein-coupled receptors, which in turn activate G-proteins to increase PLC and activate PKCs. PAR agonists also activate G-protein-coupled receptors to increase intracellular calcium. The increase in intracellular calcium causes transmitter release but also can activate a number of enzymes including $PLA_2$, CaM kinases, and PKCs. These kinases phosphorylate a number of ion channels and synaptic proteins that might further increase transmitter release.

crease SP and CGRP release from dorsal spinal cord and peripheral tissues (Steinhoff et al., 2000). Thrombin is another endogenous PAR agonist that acts at PAR1 and PAR4 receptors to produce neurogenic inflammation; however, it does not appear to increase peptide release (Steinhoff et al., 2000). Although PARs are G-protein coupled receptors that have been linked to Gq or Gi, the transduction cascades that mediate the release of peptides from sensory neurons have not been delineated. Because PAR2 agonists augment calcium entry into sensory neurons, it is intriguing to speculate that the tryptase-induced peptide release might be secondary to activation of PKC. Because PARs are localized in a number of cells in the periphery that contribute to symptoms of inflammation, further studies are clearly warranted to ascertain which inflammatory actions of the PAR agonists are through direct actions on sensory neurons.

Activation of VR1, $B_2$, and PAR receptors results in an influx of extracellular calcium and the release of calcium from intracellular stores. It is well documented that neuropeptide release is dependent on external calcium entering the cell, but the relative importance of calcium release from intracellular stores in regulating neuropeptide release is not well understood. Increasing intracellular calcium can result in activation of a number of PKC isoforms (Nishizuka, 1984; Fig. 1), which can increase neuropeptide release (Barber and Vasko, 1996). In addition, increasing intracellular calcium can activate a number of other enzymes including phospholipase $A_2$ and the calcium/calmodulin-dependent protein kinases (CaM kinases; see Fig. 1). Phospholipase $A_2$ promotes the liberation of arachidonic acid from membrane triglycerides and the production of prostaglandins that influence transmitter release (see below). Whether increasing the activity of CaM kinases in sensory neurons modifies transmitter release and neurogenic inflammation is yet to be assessed. Thus, there may be other calcium-dependent mechanisms modulating neuropeptide release.

As indicated in Table 1, not all agents that elicit currents and increase calcium entry into sensory neurons have been shown to release neuropeptides. For example, exposing small diameter sensory neurons to either ATP or acetylcholine produces inward currents through the activation of P2X or nicotinic receptors, respectively (Chen et al., 1995; Flores et al., 1996). Despite this, ATP has not been shown to directly evoke the release of neuropeptides from sensory neurons. Furthermore, it remains questionable whether exposure of sensory neurons to acetylcholine stimulates peptide release. In a similar manner, strong evidence is lacking as to whether the other agents listed in Table 1 directly stimulate transmitter release from sensory neurons. This may indicate that these agents do not directly release neuropeptides from sensory neurons under the conditions studied or, alternatively, that neuropeptides are released but at concentrations too small or over a time course too short to detect by conventional methods. Further studies are warranted to evaluate these possibilities.

It is important to note that most studies examining peptide release from sensory neurons use normal animals or use sensory neurons isolated from normal animals. It seems likely that there may be differences in the response to various agents under conditions of inflammation. Thus, chronically exposing sensory neurons to various inflammatory mediators such as neurotrophins or cytokines might result in a change in gene expression and phenotype that alters excitability. Investigations are currently underway in a number of laboratories to evaluate changes in sensory neuron excitability and transmitter release during inflammation.

## Agents That Alter Sensitivity of Sensory Neurons

The mediators listed above influence neurogenic inflammation by directly activating sensory nerve endings and increasing transmitter release. Other compounds, however, do not excite sensory neurons, but act to sensitize them. These substances are synthesized and released in response to tissue injury and have minimal or no direct effects on transmitter release from sensory neurons. Rather, they lower the threshold for firing and increase responses to suprathreshold stimuli (see Table 1). The major consequence of this sensitization of sensory neurons is an increase in the outflow of transmitters by a given stimulus and augmentation of inflammatory symptoms, especially hypersensitivity.

Of the potential sensitizing agents listed in Table 1, the proinflammatory prostaglandins are of major importance. Both prostaglandin $E_2$ ($PGE_2$) and prostaglandin $I_2$ ($PGI_2$) are released into peripheral tissues and onto the spinal cord during tissue injury or inflammation (Dirig and Yaksh, 1999) and contribute to the development of neurogenic inflammation. These prostaglandins bind to specific receptors that are localized on sensory neurons where they lower the firing threshold of these neurons (Schaible and Schmidt, 1988), increase the number of action potentials elicited by a depolarizing stimulus (Nicol and Cui, 1994) and enhance SP and CGRP release in response to capsaicin, bradykinin, and elevated potassium concentrations (Franco-Cereceda, 1989; Hingtgen and Vasko, 1994; Vasko et al., 1994).

ATP and bradykinin also have been reported to sensitize small diameter sensory neurons (Beck and Handwerker, 1974; Tominaga et al., 2001). As discussed above, ATP has not been shown to increase transmitter release directly; rather it may augment release produced by other stimuli such as capsaicin (Wu et al., 1997). Furthermore, it is now clear that not only P2X but also P2Y purinergic receptors are localized on sensory neurons, and the latter receptor subtypes do not cause depolarization of the neurons but change the sensitivity to other stimuli (Kress and Guenther, 1999; Tominaga et al., 2001). In a similar manner, bradykinin increases the firing of small diameter sensory fibers induced by noxious stimuli, potentiates heat-evoked currents in sensory neurons, and enhances proton- and capsaicin-evoked currents in cells transfected with the $B_2$ and VR1 receptors (Beck and Handwerker, 1974; Cesare and McNaughton, 1996; Chuang et al., 2001).

Evidence also points to additional compounds that are capable of sensitizing capsaicin-sensitive sensory neurons. The cytokines, interleukin-1$\beta$, interleukin-6, and tumor necrosis factor-$\alpha$ produce hyperalgesia (Watkins and Maier, 2000) and increase the heat- or capsaicin-evoked release of SP or CGRP in in vitro sensory neuronal preparations (Inoue et al., 1999; Opreé and Kress, 2000). Long-term exposure to TNF$\alpha$ also increases capsaicin currents in isolated sensory neurons (Nicol et al., 1997). The questions remain, as to the transduction cascades mediating the actions of cytokines and whether their sensitizing actions are direct or secondary to

an increase in the expression of COX2, thereby, augmenting prostaglandin production. Indeed, a number of the actions of cytokines on sensory neurons are attenuated by inhibitors of COX2, suggesting that prostaglandins may mediate these actions (Nicol et al., 1997; Inoue et al., 1999; Watkins and Maier, 2000).

There are a number of other putative sensitizing agents, although the studies defining the actions of these agents on transmitter release and neurogenic inflammation are somewhat limited. Nerve growth factor augments the excitability of isolated sensory neurons and causes hyperalgesia presumably through activation of neurotrophin receptors, which are localized on subpopulations of sensory neurons (Mendell et al., 1999). Direct evidence, however, that NGF or other neurotrophins augment peptide release at peripheral terminals of sensory neurons is yet to be revealed, although NGF does augment SP release from isolated spinal cord after C-fiber stimulation (Malcangio et al., 2000).

5-Hydroxytryptamine (5-HT) acts on capsaicin-sensitive fibers to enhance substance P-induced neurogenic inflammation (Khalil and Helme, 1990) and can increase peptide release from the isolated heart preparation (Tramontana et al., 1993). There is little evidence, however, that these actions of 5-HT are directly on small diameter sensory neurons as opposed to causing other cells to release inflammatory mediators that can alter release. Finally, nitric oxide (NO) appears to be an important mediator of vasodilatation and can cause hypersensitivity. Whether NO augments peptide release from sensory neurons as a component of its actions or affects other cells is yet to be resolved since some work suggests an action of NO on peptide release (Garry et al., 1994), whereas in sensory neurons in culture, we could not see an effect on peptide release (Dymshitz and Vasko, 1994).

## Cellular Mechanisms Mediating Sensitization of Sensory Neurons

Another important question is what are the cellular mechanisms that alter the sensitivity of sensory neurons? In recent years, this question has been the focus of much work since sensitization of capsaicin-sensitive nociceptors results in an increased release of substance P and CGRP into the periphery and a subsequent accentuation of neurogenic inflammation. In addition, attempts to diminish the symptoms of neurogenic inflammation, (especially the enhanced pain perception) using antagonists to transmitters and proinflammatory substances have met with limited success. This might be, in part, because of the multiple transmitters and proinflammatory substances that can be released from sensory neurons to subserve similar functions. It seems logical, therefore, to determine the mechanisms regulating sensitization in an attempt to prevent it rather than attenuating the actions of substances after they have been released.

To establish that a signal transduction cascade mediates sensitization of sensory neurons, several criteria need to be met. These criteria are analogous to those established by Sutherland (1972) for the involvement of a second messenger in drug action. First, the drug or hormone that produces sensitization should activate the transduction pathway. Second, activation of the transduction pathway should mimic the actions of the drug and produce sensitization. Finally, inhibiting the pathway should attenuate the actions of the drug and prevent sensitization.

To date, the above criteria have been satisfied for two pathways in sensory neurons, the cAMP pathway and the activation of PKC (see Fig. 2). The sensitizing actions of the proinflammatory prostaglandins $PGE_2$ and $PGI_2$ are mediated through the cAMP pathway (Fig. 2). Sensory neurons express a number of $PGE_2$ receptor subtypes (EP receptors) and the sensitizing action of this eicosanoid is dependent on activation of receptors coupled to Gs (Southall and Vasko, 2001). In a similar manner, $PGI_2$ acts through the IP receptor, which is also coupled to Gs. Exposing sensory neurons to $PGE_2$ or $PGI_2$ at concentrations that sensitize causes an increase in the production of cAMP (Hingtgen et al., 1995; Smith et al., 1998). Furthermore, prostaglandin-induced augmentation of stimulated peptide release is mimicked by forskolin, membrane-permeable cAMP analogs, and cholera toxin (Hingtgen at al., 1995; Smith et al., 1998). The sensitizing actions of the prostaglandins are blocked by compounds that inhibit adenylyl cyclase activity or inhibit cAMP-dependent phosphorylation (Cui and Nicol, 1995; Hingtgen et al., 1995).

The sensitizing actions of bradykinin and ATP appear to be mediated by activation of PKC (see Fig. 2). As discussed above, bradykinin augments the production of IP3 and DAG in sensory neurons (Burgess et al., 1989). Furthermore, activation of PKC with concentrations of phorbol esters that do not increase release directly or with permeable analogs of DAG augments capsaicin-evoked peptide release from isolated sensory neurons (Barber and Vasko, 1996) and increases heat-, proton-, and capsaicin-evoked currents (Vellani et al., 2001). Given that activation of PKC (but not the cAMP transduction cascade) can either excite or sensitize sensory neurons, the conditions that favor stimulation of peptide release versus augmentation of evoked release need to be determined. The response of sensory neurons to activation of PKC may be dependent on the level of activation of the enzyme, the underlying state

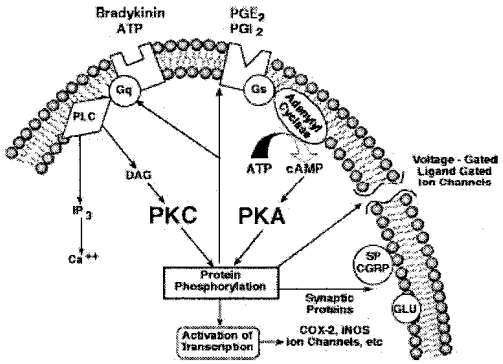


**Fig. 2.** Transduction pathways that mediate sensitization of sensory neurons. $PGE_2$ and $PGI_2$ activate G-protein-coupled receptors linked to the stimulatory G-protein, Gs. This results in an increase in adenylyl cyclase activity, enhanced production of cAMP, and activation of cAMP-dependent protein kinase (PKA). Bradykinin and ATP, activate $B_2$ and P2Y receptors, respectively, and these are linked to G-proteins that activate PLC isozymes leading to an increase in $IP_3$, and DAG. The former messenger increases calcium release from intracellular stores, whereas the latter activates PKC. Both PKA and PKC phosphorylate various proteins including ion channels and synaptic proteins that may contribute to hypersensitivity. These kinases also can increase gene transcription, which can augment the production of a number of proteins that affect excitability and transmitter release.

of the neurons, or the interaction with other transduction cascades.

There are a number of other signaling pathways in sensory neurons that are activated by the proinflammatory mediators listed in Table 1, although they have not been as extensively studied as the pathways discussed above. These pathways represent strong candidates for regulating neurogenic inflammation and require further exploration. For example, NGF binds to TrkA receptors on peptidergic sensory neurons resulting in activation of PLC and of mitogen-activated protein (MAP) kinase pathways (Ganju et al., 1998). In a similar manner, cytokines also can increase the activity of MAP kinases. This activation of MAP kinases not only influences post-translational processing but can alter gene transcription. Whether the sensitizing actions of NGF or cytokines on sensory neurons require activation of the MAP kinase pathway is yet to be established.

## Unresolved Issues and Future Directions

There remain a number of questions regarding the cellular mechanisms involved in the initiation and maintenance of neurogenic inflammation. Many transduction pathways have been examined in other cell systems and need to be studied in sensory neurons. These include (but are not limited to) the sphingomyelin pathways, the atypical protein kinases, phospholipase D, and phosphatidic acids including lysophosphatidate, and phosphoinositide 3-kinases. One example of a potential novel pathway involves the ability of phosphatidylinositol-4,5-bisphosphate ($PIP_2$) to tonically inhibit channel activity at the VR1 receptor in transfected cells (Chuang et al., 2001). This inhibition is relieved by the activation of PLC by NGF and bradykinin in a manner that is independent of PKC, suggesting an alternative way these substances can regulate neuronal excitability. These findings, however, await confirmation in actual sensory neurons.

Given the number of potential signaling pathways found in sensory neurons, it is of great interest to ascertain their role in regulating transmitter release and neurogenic inflammation. Furthermore, knowledge of the relative importance of each pathway under normal conditions or after injury and potential interactions between transduction cascades will be essential in attempts to develop therapeutic interventions to alter inflammation.

Much work also is needed to ascertain the cellular mechanisms involved in maintaining neurogenic inflammation and to determine the consequences of long-term exposure to proinflammatory mediators. One possibility is that sustained activation of sensory neurons occurs through a sequential activation of various transduction cascades that is driven by the production and release of various mediators at different times after injury. For example, neurogenic inflammation could begin with the release of transmitters secondary to acute injury. These transmitters could affect other cells in the region of injury such as degranulating mast cells to release tryptase, which could further increase peptide release. Sensitization of sensory neurons could occur with the production and release of prostaglandins and/or bradykinin at the site of injury. Subsequent chemotaxis of leukocytes into the region could result in cytokine production, which in turn could alter expression of COX2 to make more prostaglandins. Another possibility for the maintenance of neurogenic inflammation could center on the ability of various proinflammatory agents to alter gene expression in sensory neurons and thus produce proteins involved in maintaining excitability.

Another issue that has not been adequately addressed centers on the ability to turn off the inflammatory cascade. In many instances, it is not clear whether continued exposure of sensory neurons to inflammatory mediators down-regulates transduction cascades. Most studies that have been performed to date use acute administration of inflammatory mediators, despite the fact that sensory nerve terminals may be exposed to inflammatory mediators for long periods. Indeed, there is precedence for down-regulation of receptors and transduction cascades after chronic activation, but its impact on sustained neurogenic inflammation is not known. Furthermore, we are only beginning to explore the long-term changes in expression of proteins in sensory neurons that accompany sustained activation or injury. Understanding the ways of returning an inflamed region to normal is essential for understanding the etiology of neurogenic inflammation and to minimize deleterious consequences of tissue injury.

## Summary

Numerous advances in understanding the mechanisms that cause neurogenic inflammation have been achieved since the initial observations that activation of sensory neurons had physiological or pathological consequences in the periphery. We now know that release of substance P and CGRP into the periphery from small diameter sensory neurons is critical to the development of neurogenic inflammation. Additionally, we have identified a number of receptors, including VR1, $B_2$, and PARs, whose activation directly evokes the release of neuropeptides either through promoting the influx of calcium, activating protein kinase C, or disinhibition of $PIP_2$. Neurogenic inflammation also is enhanced by mediators, which sensitize sensory neurons resulting in increased neuropeptide release. Some of these, such as $PGE_2$ and $PGI_2$, produce sensitization through the activation of adenylyl cyclase while others, such as bradykinin, do so by activation of PKC. Other transduction pathways, such as MAP kinase and phophatidylinositol 4,5-bisphosphate, may also be involved in sensitization.

The work performed to date has lead to a number of drug therapies that alleviate symptoms in a number of diseases associated with neurogenic inflammation. We continue, however, to discover unique endogenous substances that act directly on sensory neurons and novel transduction cascades that regulate excitability of sensory neurons. The fundamental questions, therefore, remain as to what substances are critical for the initiation and maintenance of neurogenic inflammation and what cellular mechanisms mediate the release of proinflammatory substances from sensory neurons. Although we have come far since the discovery of the efferent function of sensory neurons, we still have a long way to go.

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